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Quantifying the Robustness of a Broth-Based *Escherichia coli* O157:H7 Growth Model in Ground Beef†

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ABSTRACT

The robustness of a microbial growth model must be assessed before the model can be applied to new food matrices; therefore, a methodology for quantifying robustness was developed. A robustness index (RI) was computed as the ratio of the standard error of prediction to the standard error of calibration for a given model, where the standard error of calibration was defined as the root mean square error of the growth model against the data (log CFU per gram versus time) used to parameterize the model and the standard error of prediction was defined as the root mean square error of the model against an independent data set. This technique was used to evaluate the robustness of a broth-based model for aerobic growth of *Escherichia coli* O157:H7 (in the U.S Department of Agriculture Agricultural Research Service Pathogen Modeling Program) in predicting growth in ground beef under different conditions. Comparison against previously published data (132 data sets with 1,178 total data points) from experiments in ground beef at various experimental conditions (4.8 to 45°C and pH 5.5 to 5.9) yielded RI values ranging from 0.11 to 2.99. The estimated overall RI was 1.13. At temperatures between 15 and 40°C, the RI was close to and smaller than 1, indicating that the growth model is relatively robust in that temperature range. However, the RI also was related ($P < 0.05$) to temperature. By quantifying the predictive accuracy relative to the expected accuracy, the RI could be a useful tool for comparing various models under different conditions.

Predictive microbial models are powerful tools for ensuring microbiological safety and quality of foods *(3, 4, 15, 21, 26)* and for helping processors comply with federal regulations. They also are useful for the effective implementation of hazard analysis and critical control point programs, microbial risk assessment *(9, 15)*, and decision support in various aspects of microbial food safety and quality *(20, 27)*.

Quantitative microbial risk assessment for the fate of pathogens in food products should include only predictive models for pathogen growth, survival, and inactivation that are valid across the entire domain of relevant products and processes *(25)*. However, experimental data and associated growth models are rarely available to account for all of the relevant variables and the range of conditions for a specific microorganism, product, and process being analyzed. Extrapolation of a microbial model to conditions not specifically tested in the original modeling experiments is fundamentally undesirable if the model is not validated for those conditions. For example, extrapolation can imply application of a model outside the original temperature domain or with different substrates (e.g., actual food products versus laboratory broth). Therefore, before a predictive model can be used for practical applications, it is important to determine its range of applicability and to understand the limits of its validity. Models always should be validated against data independent of those used to create the model. Broth-based models must be shown to accurately predict microbial response in actual foods during processing, storage, and distribution *(19)*.

The term robustness is used here to describe how well a microbial model actually predicts future events (independent of those used to create the model) relative to expectations of its performance. A model is robust when it has a broad domain of validity and accuracy comparable to that expected based on model development. Other authors have used performance evaluation *(16, 19)*, validation *(18, 24)*, and evaluation *(10, 17)* to assess model performance.

Predictive models can be validated by using subsets of the data from which the model is derived *(13)*, new laboratory data, other data from literature, or trials in industry *(16)*. Observed data and predicted values can also be compared by graphical methods or by indices of performance.

Ross *(19)* introduced two indices, the accuracy factor (A_f) and bias factor (B_f), to evaluate the performance of predictive microbial models. The accuracy factor indicates the level of confidence in the prediction of the model and is given by the equation

$$A_f = 10^{[\Sigma\,|\log(\hat{\mu}/\mu)|/n]} \quad (1)$$

where $\hat{\mu}$ and μ are the predicted and observed growth model parameters (e.g., generation time or growth rate), re-

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spectively, and n is the number of observations used in the calculation. The bias factor assesses whether the model displays any bias that could lead to fail-safe or fail-dangerous predictions and is given by the equation

$$B_f = 10^{[\Sigma \log(\hat{\mu}/\mu)/n]} \quad (2)$$

Baranyi et al. *(2)* suggested a refinement of A_f and B_f by making the mean square difference between predictions and observations the basis of A_f:

$$A_f = \exp\left[\sqrt{\frac{\sum (\ln \hat{\mu} - \ln \mu)^2}{n}}\right] \quad \text{and} \quad (3)$$

$$B_f = \exp\left[\frac{\sum (\ln \hat{\mu} - \ln \mu)}{n}\right] \quad (4)$$

They also extended the use of the modified indices by calculating the integral mean of the square differences between alternative microbial growth models under investigation over the domain of environmental factors, which enabled comparison between these models and with observations.

Several other methods for model evaluation have been reported. Wijtzes et al. *(28)* constructed plots from published generation time data of *Listeria* spp. against the corresponding predictions of a model derived from studies in laboratory broth. Predictions were evaluated, and the overall reliability of the model was examined through visual inspection of the plot. Duh and Schaffner *(13)* developed predictive equations for *Listeria* spp. growth rate based on measurements in broth. Corresponding published data on food were then added to the data set used to generate the model, and regression analysis was performed. The close similarity of the mean square error and the r^2 values of the equations fitted to either data set were taken as an indication of the reliability of the model. McClure et al. *(14)* simply computed the sum of the squares of the differences of the natural logarithms of observed and predicted values as a performance measure of predictive models. Delignette-Muller et al. *(11)* computed the relative error based on the observed values and suggested that the calculated mean absolute relative error of a model is a good indicator of prediction accuracy.

In all of these studies, however, predicted values from secondary models (e.g., growth rate or generation time) were compared with independent data for assessing predictive performance. Experimental results were not compared with predicted microbial counts, which are a product of both primary and multiple secondary models. For example, Mellefont et al. *(16)* used the indices developed by Ross *(19)* and compared the observed and predicted generation time in evaluating the performance of a square root–type model describing the growth rate of *Escherichia coli* as a function of temperature, water activity, pH, and lactic acid concentration. However, if predictive microbial growth models are to be used in risk analyses or for other applications, it is essential to know how well the complete model (i.e., primary plus secondary) predicts microbial counts, which is actually the ultimate measure of product safety.

Traditionally, predictive models have been analyzed statistically by comparing the goodness of fit to the data used to generate them *(1, 29, 30)*. Established procedures described in many textbooks *(12)* are then used to determine whether the fitted model is acceptable relative to the measurement error inherent in the data. This approach is statistically sound; however, the robustness of a microbial model relies on how well it can predict independent results and those derived under conditions that were not specifically included in the estimation of the model parameters *(15)*. This flexibility is particularly important for broth-based models, which often are used to predict microbial responses in actual food products. Therefore, the objective of this work was to propose and test a quantitative measure of robustness for predictive microbial models based on the expected and actual performance in predicting microbial populations.

MATERIALS AND METHODS

The robustness index. In this work, we propose a robustness index (RI), defined as the ratio of the standard error of prediction (SEP) to the standard error of calibration (SEC) for a given microbial model:

$$\text{RI} = \frac{\text{SEP}}{\text{SEC}} \quad (5)$$

where SEC is the root mean square error of the growth model against data used to generate the model,

$$\text{SEC} = \sqrt{\frac{\sum_{i=1}^{n_1} (y_i - \hat{y}_i)^2}{n_1}} \quad (6)$$

and SEP is the root mean square error of the model against an independent data set:

$$\text{SEP} = \sqrt{\frac{\sum_{j=1}^{n_2} (y_j - \hat{y}_j)^2}{n_2}} \quad (7)$$

where

- $\hat{y}_i$, $\hat{y}_j$ = predicted value corresponding to ith or jth data point (log CFU/g)
- y_i = value of ith experimental data point used to develop the model (log CFU/g)
- y_j = value of jth experimental data point from an independent data set (log CFU/g)
- n_1 = number of data points used to develop the model
- n_2 = number of observed data points from an independent set

There are two key features of the RI. First, it quantifies the predictive accuracy of the model (SEP) relative to the expected accuracy (SEC). Second, unlike the previous related studies *(2, 11, 13, 14, 19, 28)* where primary model parameters were used to describe a model's predictive performance, actual microbial counts from growth experiments (log CFU per gram versus time) were used in computing RI. Lower RI values (less than approximately 1) indicate a robust model, with very good accuracy against independent data (upon which the SEP is calculated), compared with the expected accuracy based on the data used in pa-

rameterizing the model (upon which the SEC is calculated). An RI was calculated for each independent data set.

The overall robustness index was also calculated to quantify overall predictive accuracy of the model relative to the expected accuracy (SEC) using the combined observed data from all independent sets in the study:

$$\text{Overall RI} = \frac{\sqrt{\dfrac{\sum_{k=1}^{N_{\text{total}}} (y_k - \hat{y}_k)^2}{N_{\text{total}}}}}{\text{SEC}} \quad (8)$$

where

N_{total} = combined total number of observed data points in all independent sets
$\hat{y}_k$ = predicted value corresponding to kth data point (log CFU/g)
y_k = value of kth experimental data point from combined independent sets (log CFU/g)

The mean relative error (RE), a variant of the value described by Delignette-Muller et al. *(11)*, also was calculated for each data set. The RE indicates whether the model, on average, displays any bias toward fail-safe or fail-dangerous predictions. The mean RE is given by the equation

$$\text{RE} = \frac{\sum_{j=1}^{n} \left(\dfrac{\hat{y}_j - y_j}{\hat{y}_j} \right)}{n} \quad (9)$$

A positive RE indicates that the model overpredicted the independent data.

Broth-based, aerobic growth model for *E. coli* O157:H7. To illustrate the proposed methodology, the aerobic growth model for *E. coli* O157:H7 found in the Pathogen Modeling Program (PMP) version 7.0 (U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, Pa.) was used as a test case. This model was parameterized using 336 sets of data (2,934 data points) *(5–8)* from laboratory media (broth) and is assumed to provide conservative estimates of pathogen growth because it was based on a pure culture system (a mixture of *E. coli* O157:H7 strains 933, 45753-35, and A9218-C1) containing high concentrations of nutrients and no competitive microbial flora *(7)*. In the present study, these original data were retrieved from ComBase (www.combase.cc). The primary model is a Gompertz equation, and the secondary models for lag phase duration and generation time are quadratic response surface models describing the effects of temperature (5 to 42°C), initial pH (4.5 to 8.5), sodium chloride concentration (5 to 50 g/liter), and sodium nitrite concentration (0 to 200 μg/ml), which were expanded from the work of Buchanan et al. *(7)*. These original broth-based data were used to calculate the SEC in this study.

Independent validation data from ground beef. A total of 132 published data sets (1,178 data points) for growth of *E. coli* O157:H7 in ground beef were used to evaluate the predictive performance of *E. coli* O157:H7 aerobic growth models from the PMP. Most of these data (124 sets), which included actual microbial log counts, were taken from records in ComBase (www.combase.cc) and from work at the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center *(22)*, and the rest (8 sets) were extracted from published studies *(21, 23, 24)*. The temperature ranged from 4.8 to 45°C, pH ranged from 5.5 to 5.9, and water activity (a_w) was assumed to be 0.99 because all data were from ground beef without additives.

RESULTS

The SEC for the aerobic growth model of *E. coli* O157:H7 in PMP 7.0 was 1.56 log CFU/g, computed by applying equation 6 to all of the original broth-based data used in developing the model. This value represented the root mean square error of the model against the broth-based data used to estimate the model parameters.

The RI values of the broth-based PMP growth model for *E. coli* O157:H7 against independent data for growth of *E. coli* O157:H7 in ground beef were calculated using equations 5, 6, and 7. These RI values ranged from 0.11 to 2.99 (Table 1). The minimum RI (0.11) was for growth experiments conducted at 8°C, pH 5.8, and water activity of 0.99. The maximum RI (2.99) was for experiments conducted at 4.8°C, pH 5.8, and water activity of 0.99. The overall RI (equation 8), computed for the aggregate of all the independent data, was 1.13.

Values of the mean RE, computed using equation 9, ranged from −0.50 to 0.52 (Table 1). Of the total independent data sets considered in this study, 63% yielded negative RE values, which indicate underprediction by the model, and the rest of the data sets yielded positive RE values, indicating overprediction against independent data.

Figures 1 and 2 include example growth curves from the broth-based PMP model predictions and the actual microbial log counts for growth of *E. coli* O157:H7 in ground beef. Figure 1 illustrates a case (RI = 1.19) where the model did not provide an accurate prediction of the data from beef under the given growth conditions. A relatively robust prediction of the PMP model is shown in Figure 2, where RI = 0.52 for the given experimental conditions, although most of the independent data are outside the 95% confidence interval (CI). Conceptually, the lower and upper limits or bands of the 95% CI are the predicted values ± (1.96 × SEC); however, this definition does not apply to the output from the PMP. The 95% CIs provided by the PMP (as illustrated in Figs. 1 and 2) reflect only the uncertainty in the secondary models and therefore do not include the original experimental error or the inherent uncertainty in the primary model. In contrast, the SEC (which is reflected in the RI) is based on the total uncertainty of the model prediction (which includes experimental error, primary model error, and secondary model error).

An analysis of variance indicated that temperature significantly affected ($\alpha = 0.05$) the RI in this study (Fig. 3). At temperatures between 15 and 40°C, the RI was close to and less than 1, suggesting that the *E. coli* O157:H7 aerobic growth model from PMP is relatively robust under these conditions for predicting *E. coli* O157:H7 growth in ground beef, although the RE in this range indicated underprediction (Fig. 4). Overall, examination of the 1,178 data points (i.e., individual observations of microbial counts) from 132 data sets (Fig. 5) also indicated that the model generally underpredicted the data from ground beef.

TABLE 1. *Robustness index (RI) and mean relative error (RE) of the PMP broth-based aerobic growth model for* E. coli *O157:H7 using data for ground beef under various experimental conditions*

Source	ComBase keycode	Strain	Temp (°C)	pH	RI	RE
ComBase	EcGB_5_1	Mixed	4.8	5.8	2.99	0.52
ComBase	EcGB_5_2	Mixed	4.8	5.8	2.91	0.49
ComBase	EcGB_5_3	Mixed	4.8	5.8	2.97	0.49
ComBase	EcGB_6_9a	DB1358	6	5.8	0.70	0.08
ComBase	EcGB_6_9b	DB1358	6	5.8	0.71	0.08
ComBase	EcGB_6_10a	GFP80EC	6	5.8	1.60	0.19
ComBase	EcGB_6_10b	GFP80EC	6	5.8	2.11	0.24
ComBase	EcGB_6_1a	OB1340	6	5.8	2.57	0.35
ComBase	EcGB_6_1b	OB1340	6	5.8	2.55	0.37
ComBase	EcGB_6_5a	OB1423C	6	5.8	2.31	0.31
ComBase	EcGB_6_5b	OB1423C	6	5.8	1.56	0.22
ComBase	EcGB_6_6a	OB1514CI	6	5.8	1.86	0.23
ComBase	EcGB_6_6b	OB1514CI	6	5.8	1.92	0.21
ComBase	EcGB_6_2a	OB90520A	6	5.8	1.51	0.21
ComBase	EcGB_6_2b	OB90520A	6	5.8	0.78	0.09
ComBase	EcGB_6_7a	OB1680G	6	5.8	1.40	0.13
ComBase	EcGB_6_7b	OB1680G	6	5.8	1.59	0.16
ComBase	EcGB_6_8a	OB1533A	6	5.8	0.94	0.09
ComBase	EcGB_6_8b	OB1533A	6	5.8	1.45	0.19
ComBase	EcGB_6_4a	OB1525C	6	5.8	1.59	0.24
ComBase	EcGB_6_4b	OB1525C	6	5.8	1.50	0.22
ComBase	EcGB_6_3a	OB141412	6	5.8	1.56	0.21
ComBase	EcGB_6_3b	OB141412	6	5.8	1.06	0.14
ComBase	EcGB_8_2a	OB90520A	8	5.8	0.32	0.04
ComBase	EcGB_8_2b	OB90520A	8	5.8	0.30	0.04
ComBase	EcGB_8_7a	OB1680G	8	5.8	0.39	0.05
ComBase	EcGB_8_7b	OB1680G	8	5.8	0.41	0.05
ComBase	EcGB_8_8a	OB1533A	8	5.8	0.52	0.07
ComBase	EcGB_8_8b	OB1533A	8	5.8	0.52	0.07
ComBase	EcGB_8_4a	OB1525C	8	5.8	0.30	0.03
ComBase	EcGB_8_4b	OB1525C	8	5.8	0.28	0.03
ComBase	EcGB_8_6a	OB1514CI	8	5.8	0.73	0.09
ComBase	EcGB_8_6b	OB1514CI	8	5.8	0.75	0.10
ComBase	EcGB_8_5a	OB1423C	8	5.8	0.13	−0.01
ComBase	EcGB_8_5b	OB1423C	8	5.8	0.11	−0.02
ComBase	EcGB_8_1a	OB1340	8	5.8	0.48	0.07
ComBase	EcGB_8_1b	OB1340	8	5.8	0.49	0.07
ComBase	EcGB_8_10a	GFP80EC	8	5.8	0.68	0.09
ComBase	EcGB_8_10b	GFP80EC	8	5.8	0.64	0.08
ComBase	EcGB_8_9a	DB1358	8	5.8	0.61	0.08
ComBase	EcGB_8_9b	DB1358	8	5.8	0.60	0.09
ComBase	EcGB_8_a	Mixed	8	5.8	1.21	0.16
ComBase	EcGB_8_b	Mixed	8	5.8	1.20	0.15
ComBase	EcGB_8_c	Mixed	8	5.8	1.21	0.17
ComBase	EcGB_8_4	Mixed	8	5.8	1.16	0.13
ComBase	EcGB_8_5	Mixed	8	5.8	1.13	0.12
ComBase	EcGB_8_6	Mixed	8	5.8	1.12	0.11
ComBase	EcGB_8_3a	OB141412	8	5.8	0.38	0.05
ComBase	EcGB_8_3b	OB141412	8	5.8	0.35	0.04
ComBase	EcGB_10_a	Mixed	10	5.8	1.19	−0.41
ComBase	EcGB_10_b	Mixed	10	5.8	1.19	−0.41
ComBase	EcGB_11_a	Mixed	11	5.8	0.69	−0.20
ComBase	EcGB_11_b	Mixed	11	5.8	0.79	−0.24
ComBase	EcGB_11_c	Mixed	11	5.8	0.70	−0.19
ComBase	EcGB_12_a	Mixed	12	5.8	1.38	−0.45
ComBase	EcGB_12_b	Mixed	12	5.8	1.34	−0.42
ComBase	EcGB_12_c	Mixed	12	5.8	1.18	−0.35
ComBase	EcGB_15_a	Mixed	15	5.8	0.92	−0.24
ComBase	EcGB_15_b	Mixed	15	5.8	0.84	−0.21

TABLE 1. *Continued*

Source	ComBase keycode	Strain	Temp (°C)	pH	RI	RE
ComBase	EcGB_15_c	Mixed	15	5.8	0.91	−0.24
ComBase	EcGB_20_a	Mixed	20	5.8	0.84	−0.22
ComBase	EcGB_20_b	Mixed	20	5.8	0.80	−0.20
ComBase	EcGB_20_c	Mixed	20	5.8	0.84	−0.21
ComBase	EcGB_20a_a	Mixed	20	5.8	0.78	−0.20
ComBase	EcGB_20a_b	Mixed	20	5.8	0.74	−0.18
ComBase	EcGB_20a_c	Mixed	20	5.8	0.78	−0.19
ComBase	EcGB_25_a	Mixed	25	5.8	0.65	−0.14
ComBase	EcGB_25_b	Mixed	25	5.8	0.66	−0.14
ComBase	EcGB_25_c	Mixed	25	5.8	0.62	−0.13
ComBase	EcGB_30_a	Mixed	30	5.8	0.53	−0.12
ComBase	EcGB_30_b	Mixed	30	5.8	0.55	−0.12
ComBase	EcGB_30_c	Mixed	30	5.8	0.52	−0.12
ComBase	EcGB_37_a	Mixed	37	5.8	0.71	−0.16
ComBase	EcGB_37_b	Mixed	37	5.8	0.74	−0.17
ComBase	EcGB_37_c	Mixed	37	5.8	0.70	−0.15
ComBase	EcGB_40_a	Mixed	40	5.8	0.90	−0.20
ComBase	EcGB_40_b	Mixed	40	5.8	1.05	−0.28
ComBase	EcGB_40_c	Mixed	40	5.8	1.07	−0.29
ComBase	EcGB_42_a	Mixed	42	5.8	1.25	−0.36
ComBase	EcGB_42_b	Mixed	42	5.8	0.91	−0.20
ComBase	EcGB_42_c	Mixed	42	5.8	1.18	−0.34
ComBase	EcGB_44_a	Mixed	44	5.8	1.40	−0.41
ComBase	EcGB_44_b	Mixed	44	5.8	1.35	−0.39
ComBase	EcGB_44_c	Mixed	44	5.8	1.56	−0.50
ComBase	EcGB_44_2a	OB90520A	44	5.8	1.08	−0.26
ComBase	EcGB_44_2b	OB90520A	44	5.8	1.19	−0.31
ComBase	EcGB_44_7a	OB1680G	44	5.8	1.17	−0.33
ComBase	EcGB_44_7b	OB1680G	44	5.8	1.25	−0.37
ComBase	EcGB_44_8a	OB1533A	44	5.8	1.29	−0.37
ComBase	EcGB_44_8b	OB1533A	44	5.8	1.33	−0.39
ComBase	EcGB_44_4a	OB1525C	44	5.8	1.26	−0.40
ComBase	EcGB_44_4b	OB1525C	44	5.8	1.23	−0.35
ComBase	EcGB_44_6a	OB1514CI	44	5.8	1.33	−0.39
ComBase	EcGB_44_6b	OB1514CI	44	5.8	1.08	−0.26
ComBase	EcGB_44_5a	OB1423C	44	5.8	1.10	−0.27
ComBase	EcGB_44_5b	OB1423C	44	5.8	0.95	−0.20
ComBase	EcGB_44_1a	OB1340	44	5.8	0.78	−0.18
ComBase	EcGB_44_1b	OB1340	44	5.8	0.69	−0.14
ComBase	EcGB_44_10a	GFP80EC	44	5.8	1.08	−0.26
ComBase	EcGB_44_10b	GFP80EC	44	5.8	1.12	−0.27
ComBase	EcGB_44_9a	DB1358	44	5.8	1.16	−0.30
ComBase	EcGB_44_9b	DB1358	44	5.8	1.21	−0.32
ComBase	EcGB_44_3a	OB141412	44	5.8	0.98	−0.24
ComBase	EcGB_44_3b	OB141412	44	5.8	1.11	−0.28
ComBase	EcGB_45_5a	OB1423C	45	5.8	1.44	−0.47
ComBase	EcGB_45_5b	OB1423C	45	5.8	1.31	−0.38
ComBase	EcGB_45_9a	OB1358	45	5.8	1.29	−0.44
ComBase	EcGB_45_9b	OB1358	45	5.8	1.33	−0.44
ComBase	EcGB_45_1a	OB1340	45	5.8	0.93	−0.28
ComBase	EcGB_45_1b	OB1340	45	5.8	1.17	−0.43
ComBase	EcGB_45_10a	GFP80EC	45	5.8	1.20	−0.35
ComBase	EcGB_45_10b	GFP80EC	45	5.8	1.43	−0.47
ComBase	EcGB_45_3a	OB141412	45	5.8	1.44	−0.46
ComBase	EcGB_45_3b	OB141412	45	5.8	1.22	−0.36
ComBase	EcGB_45_2a	OB90520A	45	5.8	1.50	−0.50
ComBase	EcGB_45_2b	OB90520A	45	5.8	1.37	−0.43
ComBase	EcGB_45_7a	OB1680G	45	5.8	1.27	−0.39
ComBase	EcGB_45_7b	OB1680G	45	5.8	1.23	−0.35
ComBase	EcGB_45_8a	OB1533A	45	5.8	1.29	−0.41

TABLE 1. *Continued*

Source	ComBase keycode	Strain	Temp (°C)	pH	RI	RE
ComBase	EcGB_45_8b	OB1533A	45	5.8	1.23	−0.37
ComBase	EcGB_45_4a	OB1525C	45	5.8	1.34	−0.41
ComBase	EcGB_45_4b	OB1525C	45	5.8	1.47	−0.47
ComBase	EcGB_45_6a	OB1514CI	45	5.8	1.41	−0.46
ComBase	EcGB_45_6b	OB1514CI	45	5.8	1.43	−0.47
Vold et al. (*23*)		Mixed	12	5.5	1.18	0.14
Vold et al. (*23*)		Mixed	12	5.5	0.93	0.03
Tamplin (*21*)		Mixed	10	5.9	0.97	−0.28
Tamplin (*21*)		Mixed	10	5.9	1.01	−0.28
Tamplin (*21*)		Mixed	10	5.9	0.86	−0.26
Walls and Scott (*24*)		Mixed	12	5.7	0.44	−0.02
Walls and Scott (*24*)		Mixed	20	5.7	0.72	−0.17
Walls and Scott (*24*)		Mixed	35	5.7	0.66	−0.13

DISCUSSION

The objective of this work was to demonstrate the application of a quantitative measure of robustness to a microbial growth model. In particular, the PMP aerobic growth model for *E. coli* O157:H7 in broth was used as a test model against published experimental data for *E. coli* O157:H7 growth in ground beef. The proposed procedure assessed the robustness of the growth model for *E. coli* O157:H7 by comparing actual microbial log counts from independent data sets with the corresponding predicted values from the model.

The RI can be interpreted as an objective measure of a microbial model's predictive accuracy based on expected performance (SEC) and actual performance (SEP) in predicting bacterial populations. If the RI is less than 1, this means that the independent data were described more accurately by the model than were the data used to create the model. An RI of zero would indicate perfect agreement between predictions and observations (regardless of the SEC).

There are two unique features of the proposed method for robustness evaluation. The first is the use of microbial log counts from an independent data set to test a predictive model. This approach is different from that of previous investigations *(2, 19),* wherein model robustness was described in terms of estimated model parameters (e.g., maximum growth rate or lag time). Although these other approaches are reasonable, they consider only a portion of the overall behavior of microbial growth (e.g., secondary model of growth rate or generation time as a function of environmental variables) and do not quantify the overall predictive capability of a model, which depends on all the model parameters and both the primary and secondary model forms.

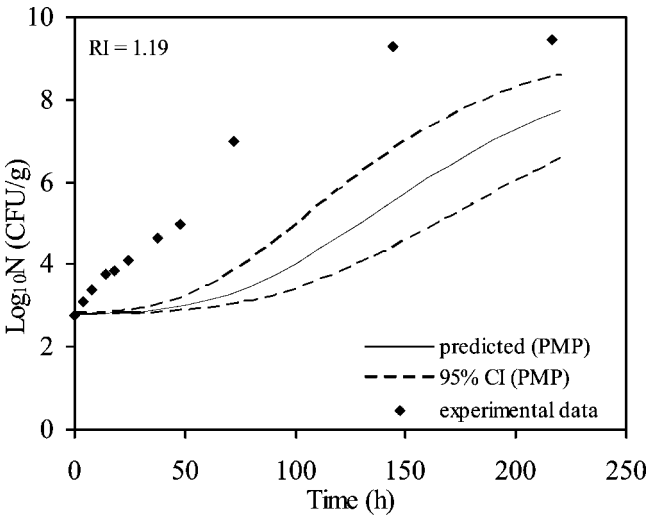

FIGURE 1. *Comparison of predictions of the Pathogen Modeling Program (PMP) broth-based growth model and observed example microbial counts for* E. coli *O157:H7 in beef at 10°C, pH 5.8, and a_w of 0.99 (broken lines indicate 95% confidence limit of prediction, as reported by the PMP).*

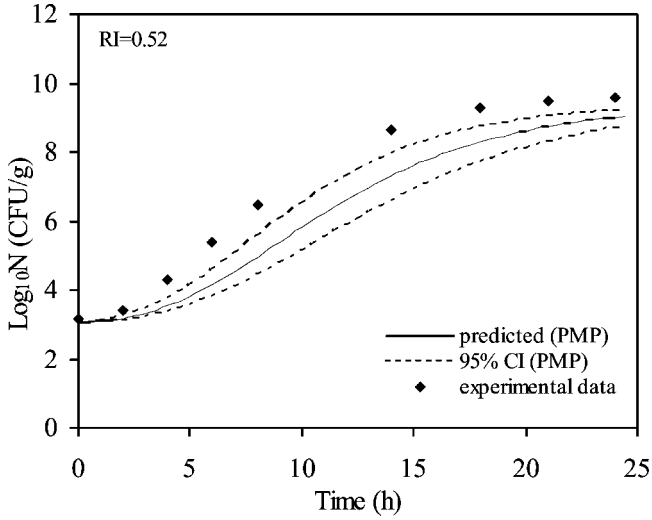

FIGURE 2. *Comparison of predictions of the Pathogen Modeling Program (PMP) broth-based growth model and example observed microbial counts of* E. coli *O157:H7 in beef at 30°C, pH 5.8, and a_w of 0.99 (broken lines indicate 95% confidence limit of prediction, as reported by the PMP).*

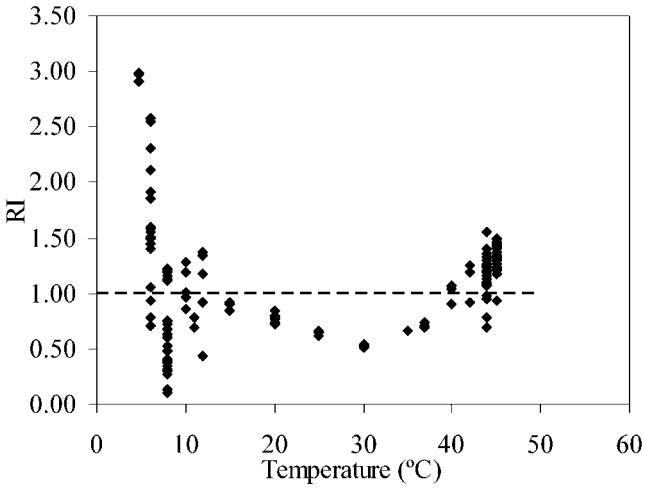

FIGURE 3. *Robustness index (RI) as a function of temperature.*

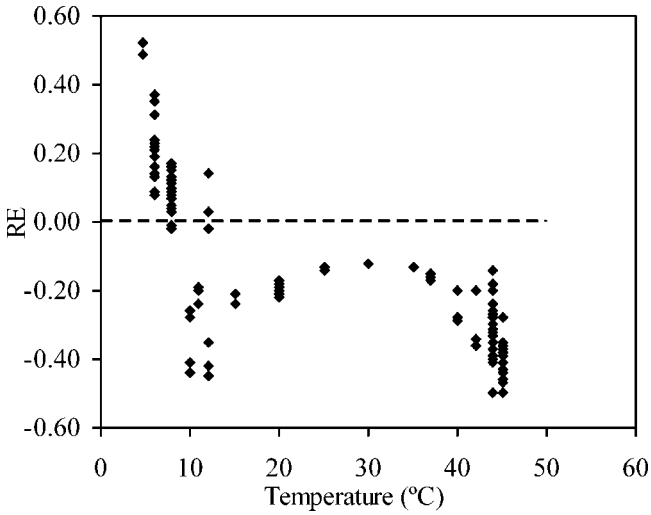

FIGURE 4. *Mean relative error (RE) as a function of temperature.*

The second unique feature of the RI is that it quantifies the predictive accuracy (SEP) of a model relative to the expected accuracy (SEC), which is derived from data used in model development. For example, consider a scenario where the growth model (model A) has an expected error (SEC) of 0.2 log CFU/g and a second growth model (model B) has an expected error (SEC) of 0.9 log CFU/g. Taking the conventional approach, model A would be judged the superior model. However, now consider the scenario where validation against independent data reveals that the predictive accuracies (SEP) of models A and B are 0.8 and 1.0 log CFU/g, respectively. Now, model B (RI of approximately 1.1) could be judged the superior model because its performance was more consistent with expectations than was that of model A (RI of approximately 4.0). If a user had selected model A based only on the better expected accuracy (0.2 versus 0.9 log CFU/g), then that user might have made decisions based on presumed confidence intervals that are (unknowingly) too optimistic, which presents a risk of fail-dangerous decisions. However, a user who selected model B would have lower expected accuracy and, therefore, would be making decisions based on a model that performs more closely to expectations (i.e., RI of approximately 1.1). In other words, for actual application of a predictive microbial model to risk analysis (or other use), indices of model performance based solely on data used to parameterize the model are insufficient for evaluating the robustness of the model.

It is commonly believed that predictive models based on monoculture microbial data measured in synthetic laboratory media overestimate the growth of microorganisms in food, and it is assumed that the media are in most cases optimal for growth. Although growth rates could be overestimated by broth-based models, for *E. coli* O157:H7 the counts can actually be underestimated. For example, if a model yields an accurate prediction of growth rate but an inaccurate prediction of lag time, then the overall predictions of microbial counts could be significantly faulty (e.g., Fig. 1). Although the RI does not indicate whether observed counts lie above or below the predicted counts, the mean RE provides this information. As defined here, negative RE values indicate that the growth model underpredicts independently observed count data. Positive values indicate an overprediction against independent data being considered. Results of this study indicate that for growth experiments conducted at temperatures >10°C, except for two cases at 12°C, the broth-based PMP model underpredicts (negative RE) the growth of *E. coli* O157:H7 in beef (Table 1 and Fig. 4). The plot of predicted versus actual microbial log counts (Fig. 5) for growth of *E. coli* O157:H7 in beef supports this claim, because most of the data points (779 of 1,178) fall below the line of equivalence.

An interesting trend in the behavior of predicted and observed log counts is shown in Figure 5. Ideally, if the points fall on the line of equivalence (line $y = x$), then the model predicts log counts perfectly. The difference between points and the line of equivalence is a measure of the inaccuracies of the respective predictions. In this study, data

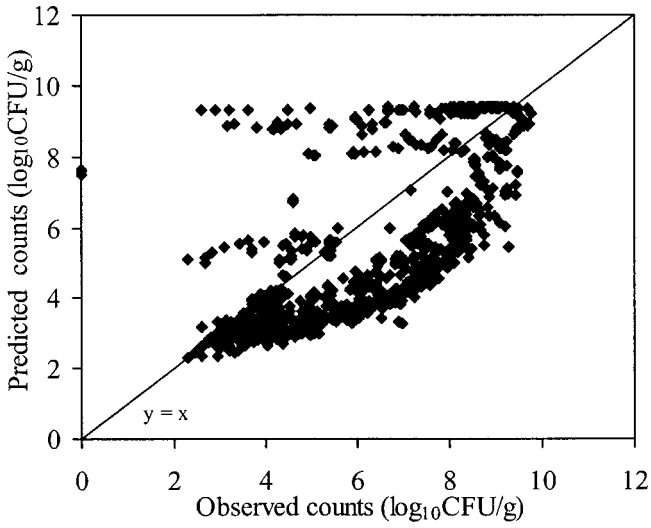

FIGURE 5. *Predicted versus observed microbial log counts of* E. coli *O157:H7 in ground beef.*

on the growth of *E. coli* O157:H7 in beef tended to form a curve that is concave upward, with lowest (initial points) and highest (end points) log counts converging toward the line of equivalence. High accuracy was observed at the starting points (which corresponds to initial microbial levels, N_0), given that these values are usually fixed and known. Reasonable accuracy was also observed toward the end points (which correspond to the asymptotic value of the Gompertz model) because these values are not significantly affected by experimental variables *(7).* Between the lowest and highest log counts, predictions were highly inaccurate, with low predicted log counts compared with actual log counts. In this intermediate range, the impact of the errors contributed by multiple model parameters (e.g., specific growth rate or lag time) are maximum, contributing to greater inaccuracies of the predicted values.

One potential use of the RI is for evaluating the effect of various experimental factors on model performance with independent data. Analysis of variance indicated a significant temperature effect ($\alpha = 0.05$) on RI in this study, which implies a problem with robustness across this domain. The plot of RI against temperature (Fig. 3) indicates that at temperatures between 15 and 40°C the RI was close to and less than 1, indicating that under these conditions the *E. coli* O157:H7 aerobic growth model from the PMP is relatively robust (although it underpredicted microbial counts in that range). RI may be related to pH, water activity, and other experimental factors (e.g., fat percentage or microbial strain). However, for this study, the ranges of these factors were too limited to make a reasonable conjecture. Information about relationships between RI and various experimental factors can have many implications for conducting reliable risk assessments.

The RI provides an objective measure of the relative robustness or predictive ability of a complete microbial growth model (primary plus secondary). The average RE provides important additional information on whether the model overpredicts or underpredicts an independent set of data. Before a model is used to predict the behavior of a pathogen under different conditions, particularly in actual food products, it is essential that the robustness of a microbial growth model be evaluated.

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